Application No.: 09/600,073 Docket No.: 21029-00205-US

REMARKS

The Office Action has been carefully considered. Applicant encloses a marked-up substitute specification as required by the Examiner. The previous Amendment (Paper No. 10) of August 22, 2003 includes the appropriate headings to the substitute specification. The original claims 1-10 were replaced by claims 11-21 in the Amendment.

In the event the office record shows 1-12 of the PCT application, the Examiner is requested to renumber amended claims 11-21 as appropriate.

Concerning the marked-up substitute specification, the addition on page 1 merely indicate the additional prior art. The frequency added to page 3 of the marked-up specification was indicated in claim 2 of the PCT application upon which the present US application is based.

Accordingly, the substitute specification includes no new matter.

Applicant previously submitted a clean copy of the substitute specification without markings.

Now that all of the Examiner's requirements have been met, entry of Applicants' last Amendment is requested.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 22-0185, under Order No. 21029-00205-US from which the undersigned is authorized to draw.

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Respectfully submitted,

By

Morris Liss
Registration No.: 24,510
CONNOLLY BOVE LODGE & HUTZ LLP
1990 M Street, N.W., Suite 800
Washington, DC 20036-3425
(202) 331-7111
(202) 293-6229 (Fax)
Attorney for Applicant

Marked Up Substitute Specification

Process for the investigation and display of tissues of human or animal origin using a high-frequency ultrasound probe

The present invention relates to a process for the investigation and display, using ultrasound echography techniques, of tissue structures of human or animal origin such as in particular the ocular globes and more particularly of the posterior segment (the vitreous cavity, the posterior wall of the globe lined by the choroid and the retina, the macula), tissue structures of the anterior segment (the cornea, the anterior chamber, the iris and the crystalline lens). The invention also relates to a device and an ultrasound probe which allow this investigation and this display to be achieved in 2D or 3D.

In ultrasound imaging and more particularly in medical echography, the choice of frequency is dictated by the compromise between resolution and penetration depth. Specifically, because of the increase in attenuation of ultrasound waves with frequency, the penetration depth of ultrasound increases with decreasing frequency. However, the image resolution decreases with decreasing frequency.

In addition, a process for the investigation and display of human tissues is known, through document US A 5,178,148, for determining the volume of a tumour or of a gland using signals coming from a probe steered by the process.

Processes are known, in particular through patent FR 2,620,327, for the investigation of ocular structures, by echography, using probes operating at low frequencies of the order of 10 MHz, and focused to a depth roughly equal to the size of an ocular globe (about 23 to 25 mm). These processes mean, on one hand, that images in section of the posterior segment of the eye can be achieved with spatial resolutions of the order of a millimetre and, on the other hand, that a

very rough examination of the entire anterior segment of the eye can be carried out.

The major drawback of low-frequency echography is mainly the low resolution (600 to 700 µm) provided by these low frequencies, which do not allow detailed analysis of the retina and the other layers of the posterior wall of the eye (choroid and sclera) and more particularly in the macular region.

In order to increase both the lateral and axial resolution, investigation and display processes using ultrasound probes at high frequency, of the order of 50 to 100 MHz (cf. US 5,551,432 and C.J. PAVLIN, M.D. SHERAR, F.S. FOSTER: "Subsurface ultrasound microscopic imaging of the intact eye", Ophthalmology 97: 244, 1990), with a short focal length (of about 4 to 8 mm), have enabled the use, with a resolution of 50 µm, of structures of the anterior segment of the eye, to depths of the order of 5 mm, or of structures of the peripheral retina which are very close to the anterior segment.

In conclusion, it is therefore accepted that the use of high frequencies seems to be limited to investigation of the anterior segment and the peripheral retina, whereas investigation of the deep structures (posterior segment) requires the use of much lower frequencies, while only providing very low spatial resolutions, of a few hundred microns.

The present invention aims to alleviate the drawbacks of the known processes of the prior art, by proposing an investigation and display process using a high-frequency ultrasound probe which combines both very high spatial resolution and a field of investigation covering the anterior and posterior segments of the ocular globe.

To this end, the process for the investigation and display of tissues of human or animal origin is characterized in that:

- an ultrasound probe is positioned, said probe being carried by a head steered by means of a three-

dimensional positioning system, in particular a system controlled by a computer at right angles to said tissue structure,

- the probe is controlled such that it generates beams of convergent high-frequency ultrasound waves whose nominal frequency is included within the range from 30 to 100 MHz with a broad bandwidth, adapted to the frequencies reflected by the structure investigated, these waves being focused on a given area of tissue structure,

- the tissue structure is scanned by the positioning system steered by the computer, while said computer carries out, in parallel, the acquisition of the signals reflected by the tissue structure,

- various signal processing operations are carried out on the data coming from the scanning, to improve the reproduction of the information and to facilitate the interpretation thereof by the practitioner.

According to another advantageous characteristic of the invention, the probe is excited such that it generates wave beams whose nominal frequency is included within the range from 30 to 100 MHz with a broad bandwidth, adapted to the frequencies reflected by the structure investigated.

According to yet another advantageous characteristic of the invention, the wave beams are focused over a vertical penetration distance of between 20 and 30 mm.

Other characteristics and advantages of the present invention will emerge from the description given hereinbelow, with reference to the appended drawings which illustrate an entirely non-limiting embodiment of the invention. In the figures:

- Figure 1 is a synoptic view of a device enabling the process forming the subject of the invention to be implemented;

- Figure 2 is a view illustrating a use of the process forming the subject of the invention for the

investigation of the posterior segment of an ocular globe;

- Figure 3 is a view illustrating a use of the process forming the subject of the invention for the investigation of the anterior segment of an ocular globe;

- Figures 4a and 4b illustrate, on one hand, a front view of one embodiment of the ultrasound probe consisting of an annular array whose focus point can be modified electronically and, on the other hand, a side view of this same probe into which a phase difference has been introduced at transmission or at reception between the various rings making up the array;

- Figure 5 is a view illustrating a use of the process forming the subject of the invention for the investigation of the anterior segment of an ocular globe, using a dynamic focusing probe;

- Figure 6 is a view illustrating a use of the process forming the subject of the invention for the investigation of the posterior segment of an ocular globe, using a dynamic focusing probe;

- Figure 7 shows a comparison between a macular section of a human globe *in vitro*, obtained by macroscopic histological imaging (right side) and an image arising from the process forming the subject of the invention (left side) where P represents the retinal folds, R the retina, S the sclera and V the vitreous humour;

- Figure 8 is the image obtained from an anterior segment of a rabbit's eye, by the process forming the subject of the invention, where C represents the cornea, I the iris, S the sclera and Cr the anterior surface of the lens.

According to a preferred embodiment of the process forming the subject of the invention, of which one system enabling its implementation is shown schematically in Figure 1, the process consists in positioning an ultrasound probe 1 mounted within a head articulated in three dimensions X, Y, Z, at least one

direction of which can be fixed, this head being steered by a servo-controlled positioning system 2, controlled by a computer 3, in particular in a direction perpendicular to the medium to be investigated.

This ultrasound probe 1 consists mainly of a transducer, in particular one made of PVDF (polyvinylidene difluoride), controlled by a transmitter/receiver 4, in order to generate beams of convergent, broadband, ultrasonic waves, these waves being able to adopt a spherical or linear profile.

Next, Figure 2 shows an investigation of the posterior segment of an ocular globe 5, previously inserted into a coupling medium 6 which does not impair the propagation of the waves, especially in the retina region. A probe 1 positioned on the pars plana 7 is used to avoid absorption of the ultrasound beam by the lens 8 (this lens also marking the boundary between the posterior segment 9 and the anterior segment 10 of an ocular globe 5). This probe 1 transmits beams of ultrasound waves set within a nominal broadband frequency range varying from 30 to 100 MHz, involving wavelengths going from 50 to 15 μm, focused at a focal length of between 20 and 30 mm and preferably 25 mm, corresponding in fact to a focus at an average depth of an ocular globe.

For example, for a probe with a nominal frequency of 50 MHz, lateral and axial resolutions of 220 and 70 μm respectively are obtained at the focal length.

The receiving system will have a bandwidth adapted to the frequencies reflected by the structure, these frequencies being lower than the transmitted frequencies because of the attenuation by the medium which is crossed.

In order to investigate the anterior segment (cf. Figure 3), this same probe 1 is used under the same control conditions as previously, in a position

offset on the vertical axis (Z axis) at a distance corresponding in fact to the previous focal length.

According to another embodiment, the focal length, especially on the vertical penetration axis, is not modified by a mechanical servocontrol 2 in the position, but by an electronic or digital device steering the probe and able to modify, by careful command, the focusing area of the probe, in order thus to obtain simultaneously a high resolution image of the anterior segment and of the posterior segment of the eye. This probe, with dynamic focusing carried out by an electronic or digital control process, consists of a multi-element probe, with circular symmetry, made up of several concentric annular transducers evenly spaced over a plane surface or with spherical concavity (refer to Figure 4a). These transducers are independent of each other and are controlled individually in transmission and in reception by pulses which are offset in time (refer to Figure 4b which shows dynamic focusing obtained by introducing a phase difference - time delay - into the transmission between the various rings).

In transmission, the generated wavefront is convergent and its curvature is modified according to the distance between the structure investigated and the probe. The peripheral rings transmit first and the excitation of the central ring is the most retarded. Thus the focal length along the axis of the probe can be varied and is therefore determined by the phase difference or the time delay introduced between the various transducers. The same principle of dynamic focusing is used in reception: the electronic delay is adjusted to the depth of the echoes which arrive at that moment at the probe. In this way the depth of field is increased without in any way degrading the lateral resolution.

A measurement system, of which each of the components (digitizer 11, computer 3, control electronics 2, transmitter/receiver 4, etc.) forming it has a bandwidth compatible with the processing and

analysis of the signals originating from the anterior segment and/or of the signals coming from the posterior segment of the eye, enables processing of the signals backscattered by the structure investigated. Thus, the backscattered ultrasound signal is amplified then digitized using the digitizer 11, at a given sampling frequency (in particular of the order of 400 MHz over 8 bits).

This same computer controls the stepper on DC motors in order to move the probe and scan the ultrasound beams over the sample in a defined step along X and along Y in order to allow another measurement point or in an R,Ω step using a probe support head which allows an arciform scan.

For *in vivo* measurements and investigations, it is necessary, in order to get round the problem of parasitic movements of the eye in its orbit, to process the signal in real time and to have available an extremely fast and accurate probe movement system.

According to another characteristic, the computer is fitted with a module for processing the image and the radiofrequency signal. This module has programmed software which enables the two quantitative approaches, of 2D and/or 3D biometry and of tissue characterization, to be carried out.

The echographic signal can be shown in real time in the form of a A-scan line or in the form of a 2D image of the B-scan type. The B-scan images can display sections in the various planes parallel to the direction of propagation of the ultrasound (cf. Figures 7 and 8). A 2D image of the C-scan type can also be calculated in order to display sections in the plane perpendicular to the direction of propagation of the ultrasound. The C-scan is able to show sections located at different depths of the whole ocular globe.

The calculation and the reconstruction of the 3D image can be carried out using programmed mathematical functions specific to the ultrasound data to be processed.

Thus, provided the propagation speed of the ultrasound in the structures investigated is known, it is possible to determine morphological characteristics of these structures, especially their thickness and/or their volume.

The processing software of the radiofrequency signal enables a frequency analysis of the digitized and recorded backscattered signals to be made in order to calculate quantitative ultrasound parameters for the purpose of tissue characterization. These parameters are in particular the attenuation coefficient in dB/cm.MHz (decibels/cm.megahertz), the overall attenuation coefficient in dB/cm, the backscatter coefficient in dB/cm.MHz and the overall backscatter coefficient in dB/cm.

These parameters can be estimated locally and their values can be shown in the form of images (parametric images).

It is of course possible to add other algorithms for processing the radiofrequency signal and the image, algorithms which could produce quantitative morphological and/or tissue information capable of characterizing the structures of the eye.

The images obtained by this investigation process, both for an ocular globe and the region of the anterior segment and the posterior segment, have a resolution which is improved by a factor of at least two to three compared with that obtained with conventional echographs and are not limited by the transparency of the media investigated as in particular with conventional optical investigation means (biomicroscopy, angiography) whose quality can be affected by the presence of cataracts and haemorrhages.

By way of example, Figure 7 illustrates the similarities between a histological image and an echographic image of the macula of a human eye (*in vitro*), and Figure 8 illustrates an image of an anterior segment of a rabbit's eye.

The process and the device which enables its implementation, such as those described previously, are not limited to applications in ophthalmology, but they can also find applications in gynaecology and obstetrics, in gastro-enterology and in the field of cardio-vascular examinations and examinations by coelioscopy, or in dermatology and more generally in any medium which reflects a usable signal.

In particular, in the field of dermatology, it is possible, using the investigation and display process forming the subject of the invention, to investigate the various thicknesses of tissue forming the skin. Thus, it is possible for example, by processing the signal, to assess the degree of skin hydration, to evaluate healing of a tissue, to localize and investigate a tumour, and finally, more generally, to to open the way to examining a large number of pathologies currently encountered in dermatology.

The focus point or focusing area of the wave beam will be adjusted within a range going from a few tenths of a millimetre to several millimetres and the waveband used will be between 30 and 100 MHz.

It is of course understood that the present invention is not limited to the embodiments described and shown hereinbefore, but that it encompasses all the variants thereof.